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EFFECTS OF ENVIRONMENTAL FACTORS ON REGENERATION AND MOLTING IN FIDDLER CRABS

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Brachyuran crabs can autotomize limbs at a preformed breakage plane in the basi-ischium, and subsequently regenerate them. The limbs grow in a folded position and at ecdysis they unfold and become functional. The progress of limb regeneration has been divided into stages by Bliss (1956). After an initial lag period, basal growth occurs which establishes the primary organization of the limb (Hodge, 1956). This may be followed by a plateau of anecdysis if environmental conditions are not suitable. The next stage, one of intensive growth and further development of existing tissues, is the proecdysial stage, culminating in ecdysis and unfolding of the new limb. A terminal plateau often occurs just prior to ecdysis. Regenerating limb buds of crabs are generally described in terms of the regeneration index, or "R-value" (Bliss, 1956), which is (limb bud length/carapace width) × 100. Use of the R-value facilitates comparisons between crabs of different sizes.

Growth of the regenerating limb is related to the molt cycle of the crab. Factors that influence the molt cycle can influence the rate of regeneration, and vice versa. The molt cycle of Brachyura has been divided into five major stages by Drach (1939). Regenerating limbs have been used as an index to the stage of proecdysis for crabs, since the growth of limb buds is closely correlated to the progress of proecdysis.

The neurosecretory and hormonal systems exert important controls over the molt cycle. For example, in some species of crabs, ecdysone will accelerate proecdysial regenerative growth, leading to molt (Passano and Jyssum, 1963). Basal growth was found to be independent of the hormone. Removal of eyestalks, which contain molt-inhibiting hormones produced by the X-organ, will lead to accelerated regeneration and early ecdysis. Removal of many limbs (multiple autotomy) will also accelerate regeneration and molting in many species of crabs (Skinner and Graham, 1972). Fingerman and Fingerman (1974) have shown that removal of as few as two limbs will accelerate ecdysis in fiddler crabs.

The neurosecretory system, which controls the molt cycle, is itself affected by the external environment. Bliss and Boyer (1964) found that in the land crab, *Gecarcinus lateralis,* darkness, moderate temperature, and solitude were necessary environmental factors to permit proecdysial growth. These factors are likewise those which would increase the crab's chances of surviving ecdysis. Light, high temperature, and the presence of another crab inhibited proecdysial growth. The presence of dry sand delayed but did not stop regeneration. Rao (1965) found that light did not inhibit proecdysial growth in the ghost crab, *Ocypode,* if crabs were on a light background, and that privacy was a critical factor only for larger crabs, smaller individuals completing regeneration and molting in the presence of

another crab. He also found that low temperature (15° C) lengthened the lag period but permitted basal growth in small individuals. Larger crabs did not initiate even basal growth. Passano (1960) found that low temperature inhibited basal growth as well as proecdysial growth in the fiddler crab, *Uca*.

The experiments described in this paper were designed to study the effects of various environmental factors, including light, starvation, privacy, temperature, salinity, and substrate on regeneration in *Uca*. In most cases regeneration was studied after multiple autotomy.

Materials and Methods

Fiddler crabs, *Uca pugilator*, were collected from Accabonac Harbor, near East Hampton, New York. In one experiment, specimens of *U. pugnax* collected from Raritan Bay, near Union Beach, New Jersey, were used. In the laboratory, crabs were maintained with a small amount of sea water in community tanks (16 × 27 cm) or in individual containers. Crabs were used in experiments within two weeks of collection.

Autotomy was induced by pinching the merus with scissors. Multiple autotomy consisted of removal of one chela and six walking legs, and was performed in all experiments but one in which autotomy consisted of removal of only the first walking leg. Groups of ten to twelve animals of approximately the same carapace width were subjected to different environmental conditions after autotomy. In group situations, individuals could be differentiated by marking the carapace with nail polish. Crabs were fed Purina "Fly Chow" twice weekly.

Limb buds were measured at regular intervals under a dissecting microscope with an ocular micrometer. In all cases the limb bud measured was that of the first walking leg, and the values obtained were converted into R_1 values. To determine the statistical significance of the data, mean R_1 values for control and experimental groups were compared by a *t*-test. Times of molting were recorded for all animals.

Results

Privacy and light

The combined effects of privacy and light were studied in a series of experiments.

Males. In a preliminary experiment five males (carapace width averaging 16 mm) kept in individual containers were compared with five in a group, all after multiple autotomy. Temperature was 19–22° C, and photoperiod was 14 hours of light to 10 hours of darkness. The results of this experiment are shown on the top of Table I. Initially, the limb buds of the isolated animals were larger than those of the grouped ones, but the difference was not statistically significant. Toward the end of the experimental period, the size of grouped animals' limb buds approximated that of the isolated ones. By day 28, two of the isolated but none of the grouped crabs had molted; but by day 30, three in each group had molted. Molting occurred at R_1 values of about 20.

The second experiment involved ten grouped and ten isolated individuals (carapace width 14–15 mm) exposed to a photoperiod of 14 hours light to 10 hours darkness (referred to as "light" below), and also ten grouped and ten isolated individuals of the same size maintained in darkness, all after multiple autotomy. The

TABLE I

R_1 values (mean ± standard error) of Uca pugilator *under differing conditions of light and privacy.*

	Days					
	7	11	14	18	21	24
Males						
Experiment 1—carapace width 16						
5 grouped	0.4 ± 0.1	1.5 ± 0.2	4.9 ± 0.3	8.9 ± 0.3	14.1 ± 0.5	17.6 ± 0.3
Isolated	0.5 ± 0.2	2.9 ± 0.7	5.8 ± 1.1	9.7 ± 1.2	14.7 ± 1.3	17.4 ± 1.1
Experiment 2—carapace width 14–15						
10 grouped light*	0.2 ± 0.1	1.7 ± 0.3	4.3 ± 0.5	8.1 ± 0.6	10.7 ± 0.7	12.9 ± 0.9
Isolated light*	0.6 ± 0.2	2.7 ± 0.5	6.7 ± 0.6	11.5 ± 0.9	15.0 ± 0.9	17.4 ± 0.7
10 grouped darkness	0.6 ± 0.2	1.9 ± 0.2	4.3 ± 0.6	9.6 ± 0.9	13.3 ± 1.0	16.5 ± 0.9
Isolated darkness	0.9 ± 0.2	2.9 ± 0.5	7.7 ± 0.7	11.1 ± 0.9	14.4 ± 1.1	16.1 ± 1.2
Experiment 3—carapace width 12–13						
Darkness		3.1 ± 0.2	10.3 ± 0.6	15.2 ± 0.8	18.9 ± 0.5	19.5 ± 0.4
Constant light		2.8 ± 0.3	10.2 ± 0.5	13.8 ± 0.8	17.2 ± 0.7	18.1 ± 0.6
Females						
Experiment 1—carapace width 15						
10 grouped	0.7 ± 0.2	2.4 ± 0.3	5.4 ± 0.5	8.6 ± 0.6	13.1 ± 1.0	16.8 ± 0.6
Isolated	0.6 ± 0.1	2.4 ± 0.3	5.6 ± 0.4	9.3 ± 0.6	13.3 ± 1.0	16.6 ± 0.9
Experiment 2—(1 leg removed) carapace width 14–15						
10 grouped light*	0.3 ± 0.1	0.6 ± 0.2	2.1 ± 0.5	5.4 ± 0.6	6.9 ± 0.8	8.7 ± 1.1
Isolated light*	0.3 ± 0.1	1.4 ± 0.3	3.2 ± 0.7	5.5 ± 0.7	7.1 ± 0.8	7.2 ± 1.0
10 grouped darkness	0.2 ± 0.1	0.8 ± 0.2	2.4 ± 0.5	4.8 ± 0.6	6.5 ± 0.9	7.7 ± 1.3
Isolated darkness	0.3 ± 0.1	1.6 ± 0.1	3.1 ± 0.5	4.7 ± 0.5	6.3 ± 0.6	6.9 ± 0.9
Experiment 3—carapace width 14–15						
10 grouped light*	1.3 ± 0.3	3.8 ± 0.5	9.8 ± 1.0	13.5 ± 1.1	17.4 ± 1.0	18.8 ± 0.7
Isolated light*	1.4 ± 0.1	5.0 ± 0.3	10.1 ± 0.6	14.4 ± 0.8	17.6 ± 0.6	19.2 ± 0.5
10 grouped darkness	1.3 ± 0.2	4.9 ± 0.2	9.4 ± 0.5	13.1 ± 0.7	16.4 ± 0.7	18.2 ± 0.4
Isolated darkness	1.0 ± 0.3	4.3 ± 0.5	9.2 ± 0.6	12.2 ± 0.8	16.1 ± 0.7	18.1 ± 0.6

* 14L/10D photoperiod

latter animals were exposed to dim light for a few minutes per week, during measurement and changing water. Results are shown in Table I. Starting at 11 days and continuing throughout the experiment the limb buds of the ten crabs in a group in light were significantly smaller than those of the isolated individuals in light. Molting was also retarded in the grouped animals in light. By 34 days 100% of the isolated but only 40% of the grouped animals had molted, an indication that grouping retarded, but did not prevent, regeneration and molting. Animals grouped in darkness initially regenerated at a rate that was not significantly different from that of grouped animals in light, but late in regeneration (about day 21) R_1 values of animals in darkness became significantly higher ($P = 0.05$). This would imply that the retarding effects of light became more evident as the crab approached ecdysis. The animals grouped in darkness molted sooner, with 90% having molted by 34 days, when only 40% of those grouped in light had molted. Animals isolated in darkness showed only a very transient period of time in which they regenerated more rapidly than animals grouped in darkness. Thus light seems to function as an inhibitory factor only in those animals that are in a group, and grouping (groups of ten but not five animals) seems to retard regeneration only in animals that are in the light. This is different from the situation in *Gecarcinus* in which the presence of another individual stopped regeneration altogether (Bliss and Boyer, 1964).

Since in this experiment "light" referred to a 14L/10D photoperiod, a third experiment was designed to ascertain if constant light could have a greater inhibitory effect, as Bliss and Boyer (1964) observed in *Gecarcinus*. A group of ten males,

carapace width 12–13 mm, was placed in an environmental chamber in constant light and with a constant temperature of 20° C, after multiple autotomy. Control specimens were kept in darkness in the chamber within a light-tight box. Results are shown in Table I. Differences in rate of regeneration became apparent only toward the end of the regeneration period (as in experiment 2). Animals in darkness molted sooner and those in light exhibited a longer terminal plateau. At 25 days, 30% of those in darkness but none of those in constant light had molted. By 31 days, 100% of those in darkness, but only 70% of those in constant light had molted. Therefore, constant light did not seem to retard regeneration and molting to any greater extent than the 14L/10D photoperiod.

Uca, therefore, unlike *Gecarcinus*, continues proecdysial growth and will molt (with slight delay) in group situations and in constant light, even though many recently molted crabs are killed and eaten by their tank-mates, especially in groups with larger sized crabs. Even large males (carapace width 19–20 mm) proceeded to ecdysis in community tanks. Crabs undergoing ecdysis in community tanks were observed to move to the opposite end of the tank from where the other crabs congregated, thus increasing their chances for survival.

Females. The preliminary experiment involved ten crabs (carapace width averaging 15 mm) in a group and ten isolated at room temperature and 14L/10D photoperiod. The results are shown in Table I. No difference was seen in regeneration rate or time to ecdysis. By 30 days 50% of the grouped and 40% of the isolated crabs had molted. Thus it seemed that privacy had no effect on regeneration and molting in female fiddler crabs.

The second experiment involved the combined effects of isolation and light. Ten grouped and ten isolated individuals (carapace width 14–15 mm) were placed in the light (14L/10D photoperiod) and ten grouped and ten isolated individuals were placed in darkness. This is the same as was done with males except that in this experiment only one leg was autotomized. The results are shown in Table I. No consistent effects of light or grouping could be ascertained. Although at 11 days the isolated crabs had regenerated more than the grouped ones in both light and darkness, this difference subsequently disappeared. The low R_1 values are a result of data both from crabs that entered proecdysis and from those individuals that reached a plateau after basal growth and whose R_1 values remained below 10. No significant difference in number of crabs that entered plateau while in light or in darkness could be observed. Crabs were maintained longer than the table shows, with no striking changes. Plateaus, observed in this experiment after single leg autotomy, were not observed in crabs after multiple autotomy, in which growth was continuous. Ovigerous females, which were not included in experimental groups, delayed growth until eggs were gone.

The third experiment was a repeat of the second, except that multiple autotomy was performed. Results are shown in Table I. Again, no consistent effects of grouping or light could be observed.

Thus, there appears to be a sex difference in effects of isolation and light on regeneration in *Uca*, isolation being a factor only for males in the light, and light being a factor only for males in a group. The retarding effects of both grouping and light were rather small.

Temperature

To test the effects of lowered temperature, ten isolated and ten grouped males (carapace width 16–17 mm) were placed in an environmental chamber at a constant temperature of 16° C and a photoperiod of 14 hours light to 10 hours darkness. There was no indication even of basal growth in any crab by three weeks. Therefore, at this time the animals were returned to room temperature and used in a salinity experiment to be described below.

To see whether lowered temperature could block further regeneration and molting in crabs already in proecdysis, several male crabs with R_1 values of 15–20 were placed in the 16° C chamber. Growth continued slowly and most of these individuals did molt within a month, showing that ecdysis can take place at temperatures too low to permit basal growth and the initiation of proecdysis.

To test the effects of elevated temperatures, ten isolated male crabs (carapace width 14–15 mm) were placed in an environmental chamber at 30° C and a photoperiod of 12 hours light to 12 hours darkness. Controls at room temperature (20–23° C) were exposed to the same photoperiod. The animals at 30° C showed a very greatly accelerated regeneration rate. By one week they had R_1 values averaging 11.2 ± 1.9, whereas controls, which were just starting basal growth, had R_1 values of 1.6 ± 0.2. By 11 days, 40% of the crabs at 30° C had molted, and by two weeks 90% had molted. At this time controls had reached R_1 values of 9.4 ± 1.7. Only after 21 days had 40% of the controls molted. The size of the regenerated limbs of the animals at 30° C was normal despite the accelerated rate of growth.

Salinity

In all previous experiments the salinity of the water was 28–30‰. To test the effects of lower salinity, a group of male crabs (carapace width 16 mm) was placed in water that had been diluted with an equivalent amount of distilled water, yielding a salinity of 14–15‰. Another group was put at one-fourth of the normal salinity (7‰). The regeneration of these crabs was compared to that of similar crabs at normal salinity. Temperature was room temperature (20–23° C) and photoperiod was 14L/10D. Results are seen in Table II. The crabs at lowered salinities were delayed in the initiation of regeneration and therefore their limb buds were smaller than those of controls, but R_1 values approached those of controls toward the end of the regeneration period. Ecdysis occurred at about the same time, with 50% of the controls, 50% of the crabs at 15‰, and 70% of the crabs at 7‰ molting by 31 days.

To test the effects of hypersalinity, the crabs removed from the 16° C chamber were used. Upon removal from the cold, one group of ten was placed in water of one and one-half times normal salinity (45‰), made by adding NaCl to the water. The other group of ten served as controls at normal salinity. They were all at room temperature and a 14L/10D photoperiod. Five days after return to room temperature regeneration had begun in both groups. The data (Table II) show that limb buds of crabs at high salinity were significantly smaller than controls at all times. Molting was also retarded. Within 25 days after removal from the cold, 90% of the controls had molted. At this time none of the crabs at high salinity had

TABLE II

R_1 values (mean ± standard error) of U. pugilator *at different salinities.*

	Days					
	8	11	14	18	21	25
Males						
Experiment 1—carapace width 16						
Sea water	0.7 ± 0.2	3.0 ± 0.3	8.0 ± 0.5	11.3 ± 0.7	15.4 ± 0.6	17.5 ± 0.6
14‰	0.2 ± 0.1	1.6 ± 0.2	6.2 ± 0.5	9.8 ± 0.7	14.6 ± 0.9	17.3 ± 0.9
7‰	0.1 ± 0.1	0.7 ± 0.3	3.9 ± 0.8	7.1 ± 1.1	13.2 ± 1.4	16.0 ± 1.6
Experiment 2—carapace width 16–17 (returned to room temperature)						
Sea water	5.1 ± 0.3	8.9 ± 1.0	14.9 ± 1.6	19.1 ± 1.0		
45‰	1.7 ± 0.6	3.3 ± 0.6	6.0 ± 1.0	9.5 ± 0.9	12.0 ± 0.9	14.6 ± 1.0
Females						
Experiment 3—carapace width 15						
Sea water	2.4 ± 0.6	9.0 ± 1.2	13.0 ± 1.3	16.9 ± 1.8	20.0 ± 0.3	
45‰	1.2 ± 0.3	6.3 ± 1.1	8.6 ± 1.0	10.9 ± 1.8	14.8 ± 1.4	15.4 ± 1.2
15‰	1.1 ± 0.6	4.3 ± 1.1	7.3 ± 1.7	10.5 ± 2.2	14.9 ± 1.6	15.8 ± 1.4

molted, but by 35 days 90% of them had molted. It is possible that this response was influenced by their past experience at 16° C.

A third experiment was performed with groups of ten female crabs (carapace width 15 mm) at normal, 15‰, and 45‰ salinity. The crabs at 15‰ regenerated more slowly at the beginning, but subsequently regenerated at a faster rate than those at 45‰ salinity (Table II). Both groups remained significantly behind controls, however. By 21 days, 50% of the controls, but only 10% of the crabs at 45‰ and 20% of the crabs at 15‰ had molted.

Food

A group of ten males (16 mm carapace width) received no food after multiple autotomy. These were compared to fed controls. All were at room temperature (20–23° C), 28–30‰ salinity, and 14L/10D photoperiod. The starved animals lagged slightly but not significantly behind controls, and they molted at about the same time. At 31 days, 50% of the controls and 40% of the unfed animals had molted. Thus, *Uca* is able to regenerate and molt regardless of food intake during the period after multiple autotomy.

Substrate

Since fiddler crabs normally live in burrows in the sand or mud, regenerating individuals in their normal substrate were compared to crabs in water alone (at room temperature, 14L/10D photoperiod, and 28–30‰ salinity). For one experiment male specimens of *U. pugnax* (carapace width 15–16 mm) were used, with mud taken from their normal salt marsh habitat, as well as ample sea water. Crabs in mud were in separate individual containers, as were controls. Crabs in the mud dug burrows for themselves, and were much slower to initiate regeneration than controls (Table III). They were also much less active than controls. Later on, however, their limb buds grew rapidly and approached the size of the control limb buds. Crabs in mud molted almost as soon as controls, with 50% of the controls and 42% of those in mud molting by 21 days. Final R_1 values of the crabs in mud

TABLE III

R_1 values (mean ± standard error) of U. pugnax *in mud and in water.*

	Days			
	7	11	14	18
Males				
Carapace width 15–16				
Water	2.6 ± 0.5	10.0 ± 0.9	15.0 ± 1.4	16.7 ± 1.5
Mud	1.1 ± 0.4	5.6 ± 1.2	10.2 ± 1.7	13.2 ± 2.2

were lower than controls, but not significantly so. Sufficient water in the mud was necessary for survival through ecdysis.

The same experiment was performed with male specimens of *U. pugilator* (carapace width 15 mm) in their normal sandy substrate. There was at no time any significant difference in size of limb buds of these crabs and controls in water, and both groups reached ecdysis at the same time.

Re-regeneration

To test the readiness of recently molted crabs to resume the molt cycle and regenerate again, some newly molted female crabs (carapace width 15 mm) in stage B2–C1 were subjected once more to multiple autotomy. In a number of these, the autotomy reflex did not occur in some limbs. These crabs were not used in the experiment. Another group was subjected simultaneously to eyestalk removal as well as multiple autotomy, to see if these two methods of accelerating proecdysis could be additive. Mortality in the latter group was very high and all had died by two and one-half weeks, although during that time their regeneration rate was faster than that of controls with multiple autotomy alone. The experiment was repeated with somewhat better survival of the destalked crabs. Crabs with multiple autotomy and eyestalk removal regenerated at a faster rate than those with only multiple autotomy (Table IV). The regeneration rate of both these groups, however, was slower than that of crabs which had not molted just prior to experimental treatment. Furthermore, about one-third of the crabs with multiple autotomy alone reached a plateau after basal growth; these were the only crabs seen in all the experiments to have a plateau of anecdysis after multiple autotomy.

TABLE IV

Re-regeneration: R_1 values (mean ± standard error) of newly molted U. pugilator *after multiple autotomy (M.A.) or multiple autotomy and destalking (D).*

	Days					
	7	11	14	18	21	24
Females						
Carapace width 15						
M.A.	0.9 ± 0.2	4.5 ± 1.0	6.8 ± 1.3	8.9 ± 1.7	11.9 ± 1.3	13.8 ± 1.7
M.A. + D	1.6 ± 0.3	5.9 ± 0.9	10.3 ± 0.9	13.2 ± 1.7	16.1 ± 1.7	

In these re-regenerating crabs, both the right and left first walking legs were autotomized, whereas initially only the right first walking leg had been autotomized. This provided an opportunity to observe whether there might be a difference in regeneration of a limb which had just previously regenerated, and one which was removed for the first time. No difference was seen, however.

Observations on regenerated limbs

In all crabs except very small individuals (carapace width under 13 mm) the regenerated limbs were somewhat smaller than the original, both in length and thickness. Furthermore, regenerated limbs were much paler in color. A microscopic examination of *U. pugilator* revealed that the newly regenerated limbs contained about the same number of black and red chromatophores as did old limbs, but that these chromatophores were different in appearance. The chromatophores in the regenerated limbs appeared elongate and sparsely branched, so that they did not give a stellate appearance, even when expanded. It is conceivable that this may be due to a decreased dispersion of the pigment, due to differential response of these new chromatophores to the hormonal milieu. It is possible that they had not yet acquired sensitivity to the chromatophorotropins. It is more likely, however, that the cells had not yet developed the elaborate branching characteristic of mature chromatophores. Another morphological basis for the color difference between old and newly regenerated limbs was revealed. By removing eyestalks, all melanophores became punctate, and it could be observed that melanophores in the old limbs had more melanin and were approximately twice as large (0.05 mm in diameter) as those in the new limbs (0.025 mm diameter). Therefore, the pale color of the new limbs was due to the presence of less pigment as well as to a less branched state of the chromatophores.

After ecdysis, crabs which had regenerated seven limbs showed no significant increase in carapace width.

Discussion

These experiments show that proecdysial growth in *Uca* is not stopped by light or grouping. These factors can only retard regeneration and delay molting somewhat under certain circumstances. Light can retard regeneration in males in a group, and grouping can retard regeneration in males in the light. The results of Bliss and Boyer (1964) with *Gecarcinus* are consistent with the ecology of that solitary burrowing land crab. *Uca*, although a burrowing crab, does emerge from burrows to forage in groups (Teal, 1958). The burrows of *Uca* frequently interconnect below the surface and they are more social crabs. It would be worth investigating whether there is a sex difference in *Gecarcinus,* since only males were used in Bliss and Boyer's (1964) study. The sex difference observed in *Uca* may be a reflection of greater aggressiveness on the part of male crabs, although an individual molting in a female community tank could get killed and eaten just as readily as one in a tank of male crabs. The response of *Uca* to light and grouping is somewhat closer to that of *Ocypode*. In that crab, juveniles were not inhibited from molting in the company of other crabs, and constant light could prolong the proecdysial period under some conditions (Rao, 1965). *Uca* is ecologically and phylogenetically more closely related to *Ocypode* than *Gecarcinus*.

The results of exposure to lowered temperatures confirm the results of Passano (1960) on *U. pugnax* after eyestalk removal, in which no regeneration took place at 15° C. He suggested that the cold blocked some metabolic processes necessary for basal as well as proecdysial growth. In *Ocypode,* Rao (1965) found that basal limb growth but not proecdysial growth could take place in juveniles at low temperatures, but adults did not even show basal growth. *Uca* is normally subjected to temperatures of 16° C and below during a large part of the year, and it might be suspected that crabs collected during the winter would be able to regenerate at low temperatures. This is not the case, however, since Passano (1960) collected crabs when the temperature was 12–15° C. The greatly accelerated regeneration at 30° C probably reflects the fact that the metabolism is higher at elevated temperatures. The initial lag period was shortened, and the rate of regeneration *per se* was greatly accelerated in these animals, which showed no signs of stress. This is in contrast to the results of Leffler (1972) on *Callinectes,* in which temperatures of 27° C and 34° C accelerated molting, but also increased the mortality rate at ecdysis. *Uca,* being an intertidal crab, would normally be exposed to warmer air temperatures during much of the summer.

The retardation of regeneration in crabs in hypo- and hypersaline water may be related to increased effort devoted to osmoregulation. It has been repeatedly demonstrated that the metabolic rate of crabs is elevated when they are subjected to unusually high or low salinities (Dehnel, 1960; Rao and Rao, 1963; Spaargaren, 1974). In the elevated temperature experiment, elevated metabolic rates were associated with accelerated regeneration and molting. Here, however, exposure to unusually high or low salinity, which also elevates metabolic rate, was associated with slower rates of regeneration. The extra energy being produced at high and low salinities was probably used for osmoregulation rather than growth. It is thought (Flemister and Flemister, 1951) that the increased metabolic rate is not due directly to increased osmotic work at the gills but to increased oxygen utilization by hydrated tissues, particularly muscle and hepatopancreas. Although salinity did not affect initiation of molting in the crab *Hymenosoma* (Broekhuysen, 1955), it has been shown to affect rates of growth and development of larval decapods, in which reduced salinity retarded growth (Costlow, Bookhout and Monroe, 1960). Metamorphosis was also delayed by high salinity (Costlow, 1967).

The lack of effect of starvation on regeneration confirms the results of Rao (1965) on *Ocypode* after limb autotomy. His experiment, however, was terminated before any crabs entered proecdysis. Apparently these species have enough food reserves to grow for that period of time without additional food intake. Roberts (1957) found that starvation inhibited molting in the crab *Pachygrapsus.* Vernberg (1959) and Wallace (1973) found that starved crabs (*Uca* and *Carcinus,* respectively) have a lower metabolic rate than fed crabs. The lowered metabolic rate which must have occurred in the present study did not have any significant effect on the regeneration rate. It is possible that starvation over a longer period of time would have had an effect.

It was initially surprising that *U. pugnax* initiated regeneration faster in water than in its own mud habitat. However, it should be mentioned that the area from which these crabs were collected is polluted, and the mud probably contained much that was deleterious to the crabs. It is also possible that toxic products accumu-

lated in the containers since they were not flushed twice daily by tides as happens in the natural environment. If the slower regeneration were due to accumulation of toxic products, one would expect that the regeneration rate would be retarded later in the regeneration period as more material accumulated. However, the delay was due primarily to a longer lag period at the beginning of regeneration. Once regeneration had commenced in the mud, it proceeded fairly rapidly, and crabs reached ecdysis at about the same time as controls. The delay in initiating regeneration was not observed in specimens of *U. pugilator* in sand. It may be worth noting that the specimens of *U. pugnax* in individual dishes in clean water regenerated faster than any similarly treated *U. pugilator*. This may indicate another species difference.

It has been shown that re-autotomizing recently molted crabs results in a slower regeneration rate, due probably to the necessity to complete the shell hardening stages before the crab can begin to prepare for the next molt. Removing eyestalks as well as limbs can accelerate regeneration somewhat, but it results in high mortality and does not produce a rate of regeneration comparable to that exhibited by crabs which had not just previously molted. The pale color of newly regenerated limbs, as compared to old limbs, has been shown to be due to a sparsely branched condition of the chromatophores and to a smaller amount of pigment contained within them. This would imply that the pigmentation of the regenerating limbs is derived from new pigment cells, rather than migration of mature pigment cells from adjacent areas. This is the mechanism believed by Goodrich and Greene (1959) to occur in repigmentation of regenerating fish fins, and by Hsiang and Brick (1969) to occur in regenerating newt limbs.

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Summary

After multiple autotomy, fiddler crabs (*Uca pugilator*) were exposed to a variety of environmental variables, during which time limb bud growth was measured and time of ecdysis was noted. The presence of other crabs retarded limb regeneration in males exposed to light. Light retarded limb regeneration in males in a group. Light and grouping had no effect on regeneration in females. Regeneration was totally inhibited at 16° C, but was greatly accelerated when the crabs were kept at 30° C. Exposure to unusually low (15‰ or 7‰) or unusually high (45‰) salinities retarded regeneration, primarily at early stages in the regenerative process. Starvation, however, had no effect on limb regeneration. Specimens of *U. pugnax* commenced regeneration more slowly in their normal mud habitat than in water alone, but *U. pugilator* regenerated at the same rate in water alone as in their normal sandy substrate. Regeneration of crabs autotomized shortly after ecdysis was much slower than that of crabs autotomized at a later stage in the molt cycle. Removal of eyestalks as well as limbs of such animals accelerated their rate of regeneration.

These effects on regeneration and molting are discussed with reference to the ecology of the fiddler crabs. The pale color of the newly regenerated limbs is due to a sparsely branched condition of the chromatophores as well as to the presence of a smaller amount of pigment within them.

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